Geranylerangylation Facilitates Proteasomal Degradation of Rho G-Proteins in Human Trabecular Meshwork Cells

Cynthia L. Von Zee¹ and Evan B. Stubbs, Jr¹,²

PURPOSE. To determine the role of posttranslational isoprenylation in regulating Rho G-protein activation and stability in human trabecular meshwork (TM) cells.

METHODS. Transformed human TM cells (GTM3) were incubated for 24 hours in the presence of activated lovastatin (10 μM) to enhance the endogenous synthesis of latent Rho proteins. Medium was replaced, cycloheximide (CHX) was added to inhibit synthesis of new proteins, and lovastatin-pretreated cells were subsequently incubated (0–24 hours) in the absence (control) or presence of farnesyl pyrophosphate (10 μM) or geranylgeranyl pyrophosphate (10 μM). Relative changes in the content of total and GTP-bound Rho G-proteins were quantified by Western immunoblot and GTP-binding ELISA, respectively. Changes in filamentous actin stress fiber organization were visualized with AlexaFluor488-conjugated phalloidin.

RESULTS. GTM3 cells cultured in the presence of lovastatin exhibited a loss of actin stress fiber organization concomitant with a marked accumulation of cytosolic inactive (GDP-bound) Rho G-proteins. Addition of geranylgeranyl pyrophosphate to the culture medium restored actin stress fiber organization while selectively facilitating the subcellular redistribution of accumulated Rho proteins from cytosol to membrane and increasing RhoA activation. Geranylgeranyl pyrophosphate selectively enhanced the degradation of newly synthesized Rho proteins. Epoxomicin, a potent and selective inhibitor of the proteasome, prevented geranylgeranyl-enhanced degradation of Rho proteins.

CONCLUSIONS. Posttranslational geranylerangylation selectively alters the lifecycle of newly synthesized Rho proteins by facilitating their membrane translocation, functional activation, and turnover. Geranylerangylation represents a novel mechanism by which active Rho proteins are targeted to the proteasome for degradation in human TM cells. (Invest Ophthalmol Vis Sci. 2011;52:1676–1683) DOI:10.1167/iovs.10-6171

In trabecular meshwork (TM) cells, the primary function of membrane-anchored Rho G-proteins is to promote filamentous actin stress fiber organization.¹ Activation of Rho signaling enhances the contractile tone of TM cells, leading to slower rates of aqueous humor (AH) outflow and higher intraocular pressure (IOP).² Inhibition of Rho proteins or downstream Rho effectors, such as Rho kinase, enhances AH outflow facility, thereby reducing IOP.³–⁸ Consequently, selective inhibitors of Rho signaling are aggressively being explored as potential therapeutic agents for the management of ocular hypertension.⁹

In humans, the Rho family of G-proteins consists of more than 20 members that serve to regulate gene expression, cell cycle progression, and organization of the actomyosin cytoskeleton.¹⁰ The Rho family of G-proteins is further categorized into eight distinct subfamilies, including Rho, Rac, and Cdc42. The Rho subfamily consists of RhoA, -B, and -C,¹¹ of which RhoA is constitutively expressed and plays an important role in regulating actin stress fiber organization and extracellular matrix synthesis in TM cells.¹²–¹⁵ By comparison, RhoB is an inducible protein that has been implicated in cell cycle arrest and stress-induced programmed cell death.¹⁶–¹⁸ In TM cells, the roles of RhoB and -C remain unclear. Diversity among Rho family members is derived, in part, from variations in their subcellular distribution. Whereas a small fraction of Rho proteins localize to the plasma membrane, the majority of RhoA and -C are cytosolic, while RhoB associates with the early endosomal/prelysosomal compartment.¹⁹ Localization of Rho to target subcellular compartments is largely dictated by lipid modifications, including posttranslational isoprenylation.²⁰

Catalyzed by farnesyl transferase or geranylerangyl transferase-I, the posttranslational isoprenylation of Rho G-proteins involves the addition of either a 15-carbon farnesyl or a 20-carbon geranylerangyl isoprenoid, respectively, to the terminal -CAAX motif of these small monomeric GTPases.²¹ Within the Rho subfamily, RhoA and -C are exclusively geranylerangylated, whereas RhoB can accept either farnesyl or geranylerangyl isoprenoids.²² Collectively, posttranslational isoprenylation serves to enhance the hydrophobicity of an otherwise hydrophilic soluble protein, facilitating association with target membranes and subsequent activation.²³ Statins, widely used for the treatment of hypercholesterolemia,²⁴ are now recognized as effective indirect inhibitors of Rho signaling.²⁵ By inhibiting HMG-CoA reductase, statins block endogenous synthesis of farnesyl- and geranylerangyl isoprenoids and thereby disrupt posttranslational isoprenylation of proteins, including Rho.²²–²⁵,²⁶ As a consequence, Rho G-proteins remain compartmentalized to the cytosol in an inactive (GDP-bound) state.²⁷ Some immature forms of Rho, however, may maintain partial function.²⁸–²⁹ These and other studies have generated considerable interest in evaluating statins as an adjunctive therapeutic strategy for the management of a wide variety of neurologic diseases, including vision disorders such as glaucomatous neuropathy.³⁰–³⁶

We recently reported that statins elicit a marked accumulation of Rho proteins in the cytosol of human TM cells, in part by enhancing expression of Rho G-protein isoforms.²⁵ In this

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study, we examined the functional consequence of posttranslational isoprenylation in regulating Rho G-protein activation and stability in human TM cells. Posttranslational geranylgeranylation was found to selectively target Rho proteins for proteasomal degradation, representing a novel function of posttranslational protein isoprenylation in human TM cells.

**MATERIALS AND METHODS**

**Human TM Cell Culture**

SV40-transformed human TM cells from a male glaucoma patient (GT3M) were the generous gift of Abbott F. Clark (Alcon Laboratories, Fort. Worth, TX). Transformed human GT3M cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) containing 4 mM of a cell culture nutrient mixture (GlutaMAX-I; Invitrogen-Gibco, Carlsbad, CA) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. GT3M cell cultures were maintained at 37°C under a humidified atmosphere of 5% CO₂/95% air.

**Treatment of TM Cell Cultures**

Confluent cultures of human GT3M cells were pretreated for 24 hours with activated lovastatin (10 µM) to enhance endogenous accumulation of Rho proteins. Where indicated, control cultures received pretreated cells cultured for an additional 24 hours in fresh media containing cycloheximide (CHX, 5 µg/mL), a selective protein synthesis inhibitor. To evaluate the effect of exogenous isoprenoids on Rho protein stability, CHX-containing media were supplemented with a commercial cocktail of protease inhibitors and solubilized by gentle homogenization in a commercial cocktail of protease inhibitors (Roche Applied Science, Indianapolis, IN). Freshly prepared lysates were subjected to either subcellular fractionation or aliquots were subjected to either subcellular fractionation or aliquots were subjected to immunoblot analysis.

**Rho protein translocation studies were conducted using lovastatin-pretreated cells that were washed once and cultured for an additional 0 to 6 hours in fresh media supplemented with l-phenylalanine (5 mM, the immediate downstream product of HMG-CoA reductase), farnesyl pyrophosphate (FPP, 10 µM), or geranylgeranyl pyrophosphate (GGPP, 10 µM), intermediate isoprenoid metabolites of the cholesterol biosynthetic pathway. In some translocation experiments, pretreated cells were cultured in the presence of geranylgeranyl transferase inhibitor (GGTI-298 (10 µM), an inhibitor of geranylgeranyl transferase-1.

To determine the stability of Rho proteins, lovastatin-pretreated cells were washed once and cultured for an additional 0 to 24 hours in fresh medium containing cycloheximide (CHX, 5 µM), an inhibitor of protein synthesis. To evaluate the effect of exogenous isoprenoids on Rho protein stability, CHX-containing media were supplemented with FPP (10 µM), GGPP (10 µM), or GGTI-298 (10 µM, 24 hours). In some experiments, lovastatin remained in the culture media to maintain suppression of endogenous isoprenoid synthesis. The role of the proteasome in Rho protein degradation was assessed by using lovastatin-pretreated cells cultured for an additional 24 hours in fresh media supplemented with CHX, GGPP, and epoxomicin (10 µM), a selective cell-permeable proteasome inhibitor.

**Immunoblot Analysis**

First, biological samples were harvested for protease inhibitors and solubilized by gentle homogenization in 10 mM Tris-HCl buffer (pH 7.5) containing 0.15 M NaCl, 1% Triton X-100, 0.5% sodium deoxycholate and 0.1% SDS. The solubilized membrane fraction was centrifuged at 15,000g for 60 minutes to obtain a clarified supernatant. The resultant clarified membrane-soluble fraction was stored at −80°C until use. Protein concentrations in cell lysates and prepared subcellular fractions were determined by the BCA method (Pierce, Rockford, IL) using bovine serum albumin as the standard.

**Rhoa Activation Assay**

The content of active (GTP-bound) Rhoa protein in GT3M cell lysates was determined by ELISA using a commercially available Rhoa-specific activation assay kit (G-LISA; Cytoskeleton, Inc., Denver, CO). Briefly, cell lysates (200 µg protein) were incubated for 30 minutes at 4°C in microtiter wells precoated with an RBD domain of Rho family effector proteins. Washed wells were incubated for 45 minutes at 23°C in the presence of a 1:10,000 dilution of mouse anti-Rhoa monoclonal antibody, followed by washing in 0.01% ethanol as a vehicle. Before use, inactive lovastatin pro-drug (100 µM). Treated cells were fixed for 15 minutes at 23°C in the presence of 1:250 dilution of mouse anti-Rhoa antibody followed by incubation with a 1:62.5 dilution of horseradish peroxidase-conjugated goat anti-mouse IgG (1:10,000 dilution) secondary antibody, and ECL detection. Relative changes in Rhoa or -B protein content were quantified by densitometry and normalized to total GAPDH content.

**Filamentous Actin Organization**

GT3M cells were cultured on chambered coverslips (Nunc Laboratory-Tek II; Thermo Fisher Scientific, Rochester, NY) and pretreated for 24 hours with vehicle (0.01% ethanol) or activated lovastatin (10 µM). Lovastatin-pretreated cells were washed and subsequently incubated for an additional 1 to 5 hours in the presence of vehicle (0.05% methanol) or GGPP (10 µM). Treated cells were fixed for 15 minutes at 25°C by immersion in phosphate-buffered (pH 7.4) 4% paraformaldehyde. Filamentous actin stress fiber organization was determined by immunofluorescence with AlexaFluor 488-conjugated phallolidin, as described elsewhere. Confocal images were captured using identical settings. Fluorescence intensity of phalloidin-positive cells was semi-quantified by using ImageJ (v1.43u; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD; available at http://rsb.info.nih.gov/ij/index.html) and the results expressed as background-corrected integrated fluorescence density.

**Statistical Analysis**

Unless otherwise specified, results are expressed as the mean ± SD of triplicate cultures, repeated at least one additional time. Statistical...
Posttranslational Geranylgeranylation Facilitates Subcellular Redistribution of Rho G-Proteins

Human GTM3 cells pretreated with lovastatin for 24 hours exhibited a marked increase in soluble (cytosolic) RhoA and RhoB G-protein accumulation compared with vehicle-treated controls (Fig. 1). As has been reported, the response to lovas- 
statin was not unique to the transformed phenotype of these cells, nor was it due to their glaucomatous origin.27 The content of RhoA and -B G-proteins associated with the particulate subcellular fraction (crude membrane preparation), however, was below the level of ECL detection and remained unaltered by lovastatin treatment. Chasing lovastatin-pretreated cells with media alone had no measurable effect on the content or subcellular distribution of accumulated cytosolic RhoA G-protein. In contrast, the content of accumulated cytosolic RhoB G-protein steadily declined in a manner that could not be accounted for by redistribution to the particulate subcellular fraction (Fig. 1A). Chasing pretreated cells with medium supplemented with f-mevalonate, the immediate downstream product of HMG-CoA reductase, elicited a detectable decline in accumulated cytosolic RhoA G-protein content (Fig. 1B). Under these conditions, cytosolic RhoA G-proteins partly distributed to the membrane fraction where they remained associated for up to 6 hours. The content of accumulated cytosolic RhoB G-proteins similarly declined in response to f-mevalonate, although at an accelerated apparent rate compared to RhoA (Fig. 1B). Cytosolic RhoB G-proteins also partly redistributed to the membrane fraction. However, in contrast to RhoA G-proteins, RhoB G-proteins were only transiently associated with the membrane (Fig. 1B).

Newly synthesized RhoA G-proteins are geranylgeranylated, whereas latent RhoB G-proteins can accept either farnesyl or geranylgeranyl isoprenoids.22 To determine the role of isoprenylation in regulating Rho G-protein subcellular distribution in human TM cells, we chased lovastatin-pretreated cultures with GGPP or FPP. Chasing pretreated cells with GGPP elicited a measurable decline in the content of RhoA and RhoB with partial redistribution to the membrane fraction (Fig. 1C), similar to that seen in the presence of f-mevalonate. Chasing lovastatin-pretreated cells in the presence of FPP+GGTI-298, an inhibitor of geranylgeranyl transferase I, completely prevented the degradation or subcellular redistribution of accumulated cytosolic Rho G-proteins (Fig. 1D).

significance was determined with one-way ANOVA followed by a Bonferroni or Dunnett multiple comparison post hoc test. In all cases, \( P < 0.05 \) was considered statistically significant.

RESULTS

Posttranslational Geranylgeranylation Facilitates Subcellular Redistribution of Rho G-Proteins

FIGURE 1. Geranylgeranylation selectively facilitates subcellular redistribution of Rho G-proteins. Western immunoblots of RhoA and RhoB proteins expressed in soluble (cytosolic) and particulate (crude membrane) fractions prepared from pooled lysates of GTM3 cells. Confluent cultures (\( n = 3 \)) were pretreated (24 hours) with vehicle (V, 0.01% ethanol) or lovastatin (10 \( \mu \)M). Lovastatin-pretreated cells were washed and cultured for an additional 0 to 6 hours, as indicated, in (A) medium alone or in medium supplemented with (B) f-mevalonate (5 mM), (C) GGPP (10 \( \mu \)M), or (D) FPP (10 \( \mu \)M)+GGTI-298 (10 \( \mu \)M). Data shown are representative of results in two independent experiments. Levels of GAPDH are shown for comparison as loading controls.

FIGURE 2. Geranylgeranylation facilitates endogenous recovery of active RhoA. (A) Confluent cultures were pretreated (24 hours) with vehicle (Veh, 0.01% ethanol) or lovastatin (Lov, 10 \( \mu \)M) and the content of GTP-bound RhoA in cell lysates was quantified by ELISA. Data shown are the mean ± SD (\( n = 6 \)) from two separate experiments each performed in triplicate. (B) Lovastatin-pretreated cells were washed and cultured for an additional 0 to 6 hours, as indicated, in fresh media supplemented with vehicle (Veh, 0.5% methanol) or GGPP (10 \( \mu \)M). Data are the mean ± SD from a single experiment performed in triplicate. * \( P < 0.01 \); ** \( P < 0.001 \), one-way ANOVA with the Bonferroni multiple-comparison post hoc test.
Effect of RhoA Reactivation on Stress Fiber Organization

By inhibiting HMG-CoA reductase, statins limit the endogenous synthesis of isoprenoids needed for membrane localization and activation of Rho G-proteins. Compared to vehicle control, GTM3 cells cultured for 24 hours in the presence of lovastatin exhibited a significant decrease in the content of GTP-bound (active) RhoA G-protein (Fig. 2A). Removal of lovastatin elicited a time-dependent restoration of GTP-bound RhoA that was significantly enhanced, compared with vehicle controls, by GGPP (Fig. 2B). These findings are consistent with GGPP-facilitated redistribution to cell membranes (Fig. 1C).

Studies have shown that Rho G-proteins regulate organization of filamentous actin stress fibers. To determine whether
Geranylgeranylation Selectively Targets Rho G-proteins for Proteasomal Degradation

In addition to facilitating the subcellular redistribution and functional reactivation of Rho G-proteins, we examined the possibility that geranylgeranylation may selectively alter Rho G-protein stability in human TM cells. Lovastatin-pretreated GTM3 cells washed and chased in the presence of CHX, an inhibitor of protein synthesis, exhibited a steady decline in accumulated RhoA (Fig. 4A) and RhoB G-proteins (Fig. 4B). The band densities of Rho shown in Figures 4A and 4B were quantified, normalized to GAPDH staining, and used to calculate changes in RhoA and RhoB protein half-life, by using a best-fit exponential decay analysis. The apparent half-life (t½) of RhoA G-proteins in GTM3 cells is approximately 13.2 hours. By comparison, RhoB G-proteins are less stable, exhibiting an apparent half-life of 6.7 hours. Interestingly, as shown in Figure 4, leaving lovastatin in the media extended the half-life of both RhoA (t½ = 19.4 hours) and RhoB (t½ = 11.2 hours). A similar increase in Rho G-protein stability was observed when GGTTI-298 (10 μM) alone was present in the chase media (data not shown). Addition of GGPP (but not FPP) to the lovastatin-containing chase media, however, selectively facilitated a decline in RhoA and RhoB G-protein content (Fig. 4C). The content of GAPDH, a constitutively expressed housekeeping enzyme, remained unaltered throughout these experiments.

The mechanism by which geranylgeranylation facilitates Rho G-protein reactivation in TM cells elicits functional consequences, we semiquantified restoration of filamentous actin stress fiber organization after removal of lovastatin. Vehicle-treated GTM3 cells express numerous phalloidin-positive filamentous actin stress fibers (Fig. 3A, Vehicle). In contrast, cells pretreated with lovastatin exhibited a marked reduction in filamentous actin stress fiber staining along with qualitative changes in cell morphology (Fig. 3A, Lovastatin). Removal of lovastatin produced a modest qualitative (nonsignificant) increase of phalloidin-positive filamentous actin stress fiber staining that was significantly enhanced at 6 hours by GGPP (Fig. 3B). These findings are consistent with GGPP’s facilitating cytosol-to-membrane relocalization (Fig. 1C) and functional reactivation (Fig. 2B) of Rho.

**FIGURE 4.** Geranylgeranylation selectively facilitates degradation of Rho G-proteins. Western immunoblots of (A) RhoA and (B) RhoB proteins expressed in cell lysates prepared from GTM3 cells. Confluent cultures were pretreated for 24 hours withLovastatin (10 μM). Pretreated cells were cultured (chased) for an additional 0–24 hours in fresh medium containing 5 μM CHX (Media) or were allowed to remain in lovastatin-containing medium in the presence of 5 μM CHX supplemented without (Control) or with 10 μM GGPP, as indicated. Data shown are results from a single experiment performed in duplicate. Levels of GAPDH are shown for comparison as loading controls. (C) Quantitative comparison of RhoA (top) and -B (bottom) content in lovastatin-pretreated GTM3 cell lysates before (0 hours) and 24 hours after the indicated chase. Data are the average GAPDH-normalized band densities from a single experiment resolved on a single immunoblot, representative of two separate experiments.
Phosphorylation, palmitoylation, isoprenylation, and ubiquitination are considered key posttranslational events that regulate the lifecycle of many intracellular proteins, including the small monomeric G-proteins such as Rho. Cyclic nucleotide-dependent phosphorylation of serine 188 on RhoA is reported to enhance the stability of this protein through RhoGDI-mediated cytoplasmic compartmentalization in vascular smooth muscle cells. Interestingly, sequestering active RhoA in the cell cytosol was found to protect against ubiquitin-mediated proteasomal degradation. In TM cells, as well as most eukaryotic cells, the ubiquitin-proteasome oligomeric complex plays an important role in regulating protein turnover. Targeting of RhoA for proteasomal degradation has been clarified, in other cell systems, and proceeds by Smurf1-catalyzed ubiquitination. Recently, Chen et al. showed that RhoA can also be ubiquitinated by Cul3, a Cullin family scaffolding protein. Knockdown of Cul3 results in abnormal actin stress fiber organization and altered cell morphology.

Whereas ubiquitination targets proteins for degradation by the 26S proteasome, other cytoplasmic proteasome assemblies (20S) have been shown to selectively degrade oxidized proteins. Cells of TM are continuously bathed in aqueous humor enriched in reactive oxygen species. It has been suggested that chronic exposure of TM cells to oxidative stress may contribute to alterations in conventional outflow pathway in primary open-angle glaucoma (POAG). A recent report from our laboratory showed that by disrupting endogenous isoprenylation, human TM cells exhibit a marked increase in the content of soluble Rho proteins, in part, through enhanced mRNA expression. Here, we address the possibility that isoprenylation, in addition to facilitating membrane translocation and activation, may serve a novel role in regulating Rho protein turnover in TM cells. It is interesting to note that degradation of oxidized proteins by the 20S proteasome initially proceeds by recognition of exposed hydrophobic amino acid surface residues. A functional consequence of posttranslational isoprenylation is enhanced hydrophobicity of an otherwise soluble protein.

A precedence for posttranslational isoprenylation in regulating turnover of Rho proteins, specifically RhoB and possibly other Rho family members, has been established. Data reported herein strongly support a selective role for geranylgeranylation, but not for farnesylation, in targeting RhoA and -B proteins to the 20S proteasome for degradation. A potential limitation of these findings resides with the use of GTM3 cells, an SV40- transformed cell line of glaucomatous origin. The GTM3 cell line, however, has been well characterized and expresses a phenotype consistent with differentiated nontransformed human primary TM cells. Furthermore, statin-dependent changes in Rho expression are not unique to cell transformation or cell origin, but rather represent a general biochemical response to inhibitors of HMG-CoA reductase. Nonetheless, a comparative assessment of Rho degradation in TM cells from healthy and glaucomatous donors is warranted to resolve this potentially useful therapeutic distinction.

The mechanism by which a 20-carbon isoprenoid selectively targets Rho proteins to an epoxomicin-sensitive proteasome in TM cells is unclear, but presumably involves specific recognition of the isoprenoid rather than a general property, such as increased hydrophobicity. This specific recognition is most evident when considering geranylgeranyl-specific turnover of RhoB, which itself is capable of accepting either a 15-carbon farnesyl or a 20-carbon geranylgeranyl isoprenoid. Compartmentalizing newly synthesized Rho proteins to the cytosol by disrupting their ability to be geranylgeranylated may represent a general strategy by which isoprenylated proteins in TM cells are protected from proteasomal degradation.

Additional proteolytic systems that may participate in regulating Rho protein turnover in TM cells include calcium-regul-

![Figure 5](https://example.com/figure5.png)

**Figure 5.** Epoxomicin protects against GGPP-facilitated degradation of Rho G-proteins. (A) Immunoblot of Rho proteins expressed in cell lysates prepared from GTM3 cells. Confluent cultures were pretreated for 24 hours with lovastatin (10 μM). Pretreated cells were cultured (chased) for an additional 24 hours in lovastatin-containing media in the presence of 5 μM CHX supplemented without or with GGPP (10 μM) and/or epoxomicin (10 μM), as indicated. Data shown are from a single experiment performed in triplicate and are representative of two independent experiments. (B) Quantitative densitometry of RhoA (top) and RhoB (bottom) results shown in (A) normalized to GAPDH and expressed as the mean ± SD. *P < 0.05; **P < 0.01; one-way ANOVA with the Dunnett multiple-comparison post hoc test.

**Geranylgeranylation Facilitates Rho Protein Degradation**

enous synthesis of GGPP, or inhibiting the transfer of this isoprenoid onto Rho proteins, elicits a loss of actin stress fiber organization in TM cells concomitant with a marked accumulation of newly synthesized inactive Rho proteins in the cell cytosol. Geranylgeranylation, but not farnesylation, of soluble Rho proteins restored actin stress fiber organization while facilitating the subcellular redistribution and functional reactivation of these small monomeric GTPases. Limiting endogenous geranylgeranylation of Rho proteins extended their half-life by nearly twofold. By comparison, GGPP selectively facilitated the degradation of RhoA and -B proteins. Epoxomicin, a potent inhibitor of the 20S proteasome, prevented geranylgeranyl-enhanced degradation of Rho proteins.
lated cysteine proteases (calpains), ATP-dependent mitochondrial proteases (e.g., Lon protease), and autophagy by lysosomal compartmentation. However, specific inhibitors of the autophagosomal/lysosomal proteolytic pathways—ammonium chloride (0–30 μM), chloroquine (0–10 μM), 3-methyladenine (0–10 mM), and wortmannin (0–10 μM)—failed to protect Rho proteins from geranylgeranyl-facilitated degradation (data not shown). By comparison, the proteasome inhibitor lactacystin (0–30 μM) was only partially successful at preventing degradation of Rho in the presence of GGPP. Lactacystin is structurally distinct from epoxomicin and covalently binds to the N-terminal threonine of the 20S proteasome subunit X and irreversibly modifies all catalytic β subunits. By comparison, the α,β-epoxeyketone epoxomicin covalently binds to and selectively inhibits the LMP7, X, Z, and MECL1 catalytic β subunits of the 20S proteasome. Collectively, the data presented herein suggest that geranylgeranylation preferentially targets RhoA and -B G-proteins in TM cells to the 20S proteasome, but not to the autophagosome or the lysosome. Proteasomal targeting of RhoB may itself be a unique characteristic of TM cells, since a recent study supports degradation of isoprenylated, palmitoylated RhoB through an endolysosomal pathway.

The functional significance of the present findings are underscored by the dominant role RhoA, and possibly RhoB, play in regulating filamentous actin stress fiber organization and cellular contractility of human TM cells. In agreement with Song et al., we have observed marked qualitative changes in TM cell shape and reduced Factin cytoskeletal organization after statin treatment. In both of these studies, the disruptive effect of statins on cell shape and Factin organization was prevented by GGPP, interfering posttranscriptional isoprenylation of Rho. In addition to changes in cell morphology, statins were found to promote marked changes in localization and expression of both RhoA and RhoB in human TM cells and significantly increase aqueous humor outflow facility. Moreover, inhibition of Rho signaling with C3 exoenzyme or expression of dominant-negative RhoA enhanced outflow in human anterior segment cultures.

Collectively, these studies support the thesis that dysregulation of Rho signaling may contribute to the development of POAG by enhancing the contractile tone of the TM with a subsequent restriction of aqueous humor outflow and compensatory elevation of intraocular pressure. The Rho signaling pathway has therefore generated much interest as a plausible target for the development of new therapeutic strategies for the management of POAG. The observation that geranylgeranylation selectively regulates the lifecycle of Rho G-proteins raises new concerns, however, that abrupt discontinuation of statin therapy may precipitate a rebound pathologic elevation of Rho-dependent restriction of AH outflow.

In conclusion, we provide evidence supporting posttranslational geranylgeranylation as a key mechanism by which newly synthesized Rho G-proteins are translocated to membranes, activated, and subsequently targeted to the 20S proteasome for degradation in human TM cells. An isoprenoid-dependent mechanism governing Rho G-protein turnover exists in GTM3 cells and may represent a novel means by which cells regulate Rho-dependent cell signaling.

References


